



## A NEW SPECIES OF *ISOPERLA* BANKS (PLECOPTERA: PERLODIDAE) FROM THE SOUTHERN APPALACHIANS, WITH NOTES ON THE *I. MONTANA* GROUP

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### ABSTRACT

A new species, *Isoperla dewalti* Verdone & Kondratieff, 2017, is described from the Appalachian Mountains of North Carolina, Tennessee, and Virginia. The new species is proposed based on details of the male aedeagus, paraprocts, vesicle, general body coloration, female subgenital plate, and uniqueness of the ovum chorion. Supporting data includes color images and scanning electron micrographs. *Isoperla smithi* Szczytko and Kondratieff, 2015 is synonymized with *I. nelsoni* Szczytko and Kondratieff, 2015 based on examination of additional material. Previously unreported records of *I. montana* (Banks, 1898) and *I. nelsoni* are presented.

**Keywords:** Plecoptera, stonefly, Perlodidae, *Isoperla dewalti* Verdone & Kondratieff, 2017, Appalachian Mountains, Great Smoky Mountain National Park, North Carolina, Tennessee, Virginia

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### INTRODUCTION

*Isoperla* Banks is the most diverse Plecoptera genus in the Nearctic Region with 84 described species (DeWalt et al. 2017). Szczytko and Kondratieff (2015) recently described 22 new species from the eastern United States, recognizing a total of 60 species of *Isoperla* with eastern Nearctic distributions. Fifty-three species were assigned to 12 species groups, while seven species were left unassigned. Their review has shed much light on the taxonomy and distribution of *Isoperla* species and has allowed for the description of additional taxa in this enigmatic genus. One new species of *Isoperla* has been described since their review (Verdone & Kondratieff 2016), with two more currently in preparation (Beaty et al. in press).

Remarkably, an additional undescribed species of *Isoperla* was recently discovered while studying the conservation status of stonefly species in the southern Appalachians of North Carolina, Tennessee, and Virginia.

The new species is a member of the *I. montana* Group which includes *I. montana* (Banks, 1898), *I. nelsoni* Szczytko & Kondratieff, 2015, and *I. smithi* Szczytko & Kondratieff, 2015 (Szczytko & Kondratieff, 2015). Males of this group are characterized by a single posterior sclerotized arrowhead shaped aedeagal spine plate with an upturned distal stout spine, dark brown bars connecting the ocelli, and moderately to heavily sclerotized paraprocts that are produced slightly over tergum ten (Szczytko & Kondratieff 2015).



Fig. 1. Adult habitus of *Isoperla dewalti* sp. n., Helton Creek Grayson Co., Virginia, 2X.

Szczytko and Kondratieff (2015) reported that males of *I. montana* and *I. nelsoni* also possessed a posteroventral membranous aedeagal tube, but were uncertain whether *I. smithi* also possessed this structure. Additionally, the ovum of *I. montana* and *I. nelsoni* are triangular in cross section, have distinct cross shaped ridges dividing anterior and posterior poles, and display hexagonal follicle cell impressions with elevated ridges. The ova of *I. smithi* was unknown at the time of description. Species belonging to the *I. montana* Group occur from Atlantic Canada south to Georgia and Alabama and west to Indiana and Kentucky (Szczytko & Kondratieff 2015).

## METHODS

Adult stoneflies were collected using a beating sheet (BioQuip, Rancho Dominguez, California, catalog # 2840C) or handpicked from substrates using forceps. Male and female specimens were kept alive in modified ventilated plastic jars (BioQuip, Rancho Dominguez, California, catalog # 8903) for 1–4 days and allowed to fully sclerotize and produce eggs. Live male specimens were

prepared under a dissecting stereomicroscope. Using wide tipped forceps, the abdomen was gently squeezed until the aedeagus was fully everted, then the specimen was fixed in position by briefly submerging it in near boiling water for 2–3 seconds. In several specimens with the aedeagus everted the abdomen was clipped and cleared in 10% Potassium Hydroxide (KOH), heated to a near boil, then promptly removed and placed in distilled water.

Color images were captured using a Canon EOS 5D digital camera with a Canon MP-E 65 mm 5X macro lens. Images are a compilation of 15–65 serial photomicrographs taken at progressively deeper focal planes using Stack Shot and controlled by Visionary Digital Passport software. Composite images were made using Zerene Stacker version 1.04. Measurements and image adjustments including background color correction, color levels and sharpening functions were accomplished using Adobe Photoshop CS6 Extended.

Male terminalia were prepared for scanning electron microscopy by removing abdomens using size 5s forceps with offset tips. Specimens were cleaned using a fine tipped paint brush and sonicated

in glass microvials filled with 80% ethanol for 30 seconds. Terminalia were serially dehydrated in ethanol in ten minutes intervals at concentrations of 80%, 90% and 95%. Further dehydration was accomplished using critical point drying. Specimens were mounted on aluminum stubs using double stick copper tape and Isopropanol graphite paint. Ova were dissected from a female specimen and cleaned in 80% ethanol using a fine tipped paint brush and sonicated for 30 seconds to remove tissue adhered to the ovum surface. They were then air dried and mounted to an aluminum stub using double stick copper tape. Terminalia and ova were sputter coated in 12 nm gold in preparation for scanning electron microscopy. Micrographs of the male terminalia and ova were taken using a JEOL JSM-6500F Field Emission Scanning Electron Microscope at the Central Instrument Facility, Imaging Laboratory, Colorado State University (<http://cif.colostate.edu/imaging-laboratory/>).

Coordinate data for new material were obtained using Topo Maps version 1.16 for iPhone. Additional material was examined from the C.P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, Colorado (CSUIC). Coordinate data for legacy specimen records were gathered using GEOLocate v. 3.22 (<http://www.museum.tulane.edu/geolocate>) and those values are indicated by “[ ]”. Coordinates were plotted and mapped using ArcMap, ArcGIS 10.4.1 for Desktop. Sub-basin boundaries (HUC8) were obtained from USGS Watershed Boundary Dataset; physiographic boundary data were obtained from USGS Water Resources NSDI Node and ESRI Canada.

Morphology and terminology follow that of Szczytko and Kondratieff (2015). Codens for depositories of specimens are: C.P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, Colorado (CSUIC), the Illinois Natural History Survey, Champaign, Illinois (INHS), and the United States Museum of Natural History, Washington, D.C. (USNM). The holotype of the new species is deposited in the INHS. Data for all specimens examined may be downloaded from a [CSV file](#).

## RESULTS

### *Isoperla dewalti* Verdone & Kondratieff sp. n.

<http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:501137>

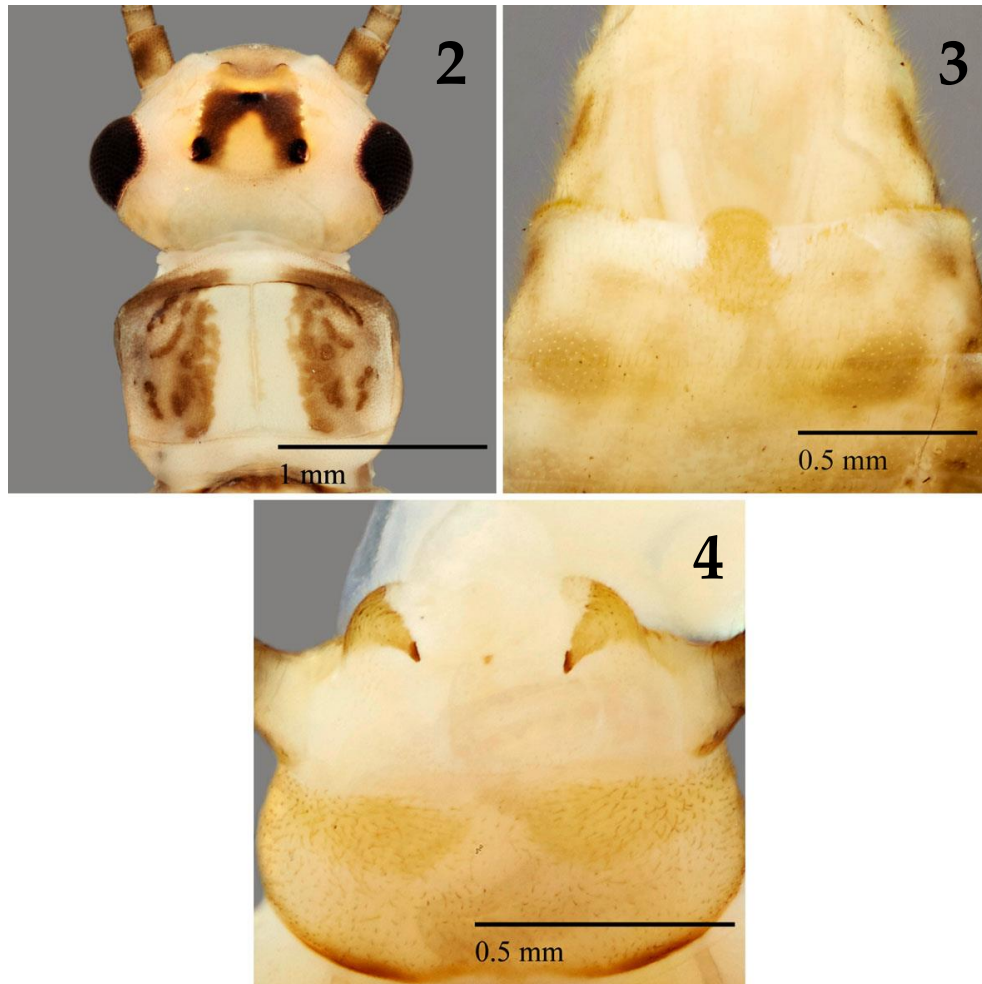
(Figs. 1–20)

**Material examined.** *Holotype* ♂ U.S.A. – Virginia: **Grayson Co.**, Helton Creek, Rte 783 at gated trail by pond, N 36.62816, W 81.56684, 13 May 2017, C. Verdone, B.C. Kondratieff (INHS, Insect Collection 659585). *Paratypes*: **North Carolina: Avery Co.**, Shantee Creek, Adams Apple Dr., SW of Foscoe, N 36.12439, W 81.82068, 13 May 2010, B.C. Kondratieff, R.E. Zuellig, D.R. Lenat, R.F. Kirchner, ♂ (CSUIC). **Macon Co.**, Tellico Creek, Tellico Rd. ~0.5 mi. W of Sugar Cove Rd., N 35.27688, W 83.54347, 15 May 2017, C. Verdone, B.C. Kondratieff, ♂ (CSUIC). **Swain Co.**, Beech Flats Prong, GSMNP, Hwy 441, N 35.60227, W 83.41534, 16 May 2017, C. Verdone, B.C. Kondratieff, ♂ (CSUIC). **Watauga Co.**, Boone Fork, CR 1561, W of Boone Fork, [N 36.12310, W 81.77006], 2 May 2005, B.C. Kondratieff, R.E. Zuellig, D.R. Lenat, R.F. Kirchner, ♂, 2♀ (CSUIC). **Tennessee: Sevier Co.**, trib. to Le Conte Creek, GSMNP, Twin Creeks Uplands Research Lab, N 35.68706, W 83.50096, 16 May 2017, C. Verdone, B.C. Kondratieff, 2♂, 2♀ (CSUIC). **Virginia, Franklin Co.**, Grassy Fork, [Co. Rd. 619, 2 mi. SE Snow Creek Rd.], [N 36.81097, W 79.74516], 21 May 2012, B.C. Kondratieff, R.F. Kirchner, ♂ (CSUIC). **Grayson Co.**, Same location as holotype, 12 May 2017, C. Verdone, B.C. Kondratieff, J. Richards, R. Agbalog, 4♂, 17♀ (USNM); Same location as holotype, 13 May 2017, C. Verdone, B.C. Kondratieff, 6♂, 71♀ (CSUIC); Same location as holotype, 17 May 2017, C. Verdone, B.C. Kondratieff, ♀ (INHS, Insect Collection 659584).

**Distribution.** U.S.A. – NC, TN, VA (Fig. 22).

**Etymology.** The new species is named in honor of Dr. R. Edward DeWalt, Illinois Natural History Survey, Champaign, Illinois, for his dedication and numerous contributions to the study of stoneflies. The proposed common name is the “DeWalt Stripetail”.

**Male.** Macropterous; forewing length 10.6–11.3 mm

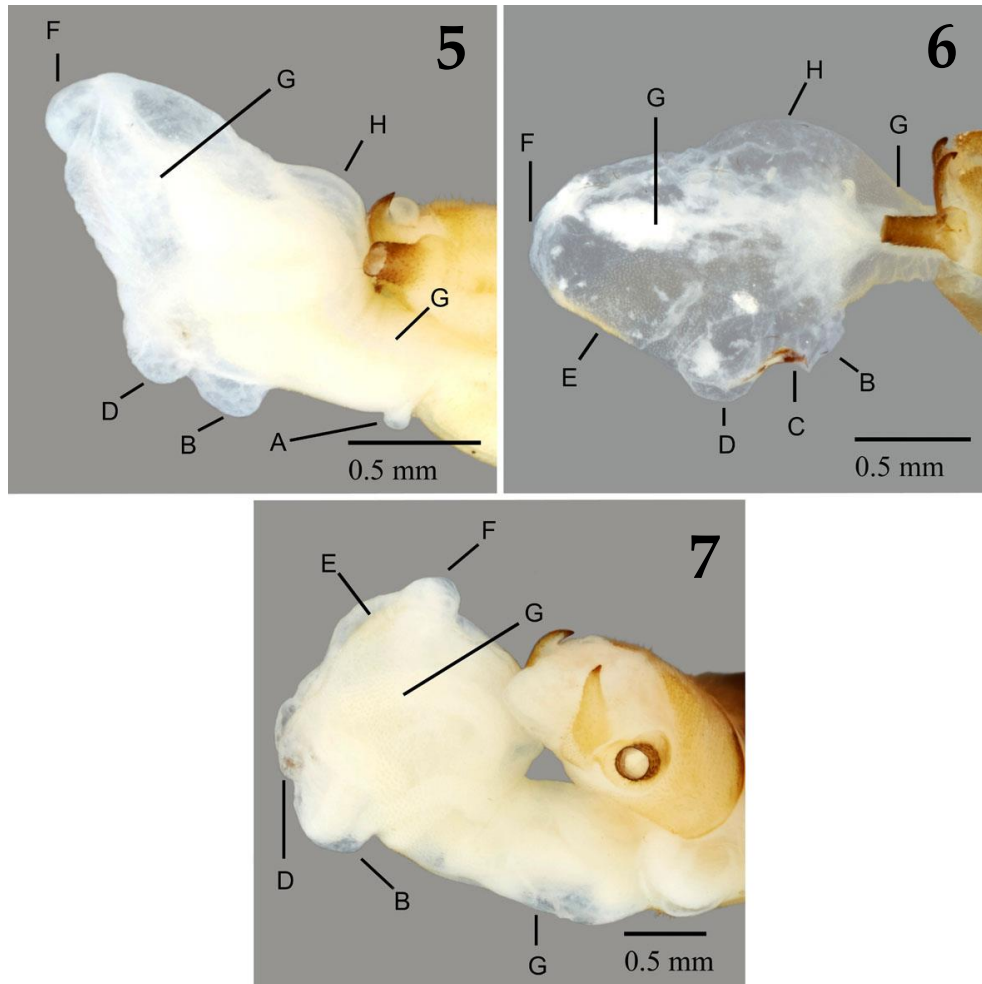


Figs. 2–4. *Isoperla dewalti* sp. n., Helton Creek, Grayson Co., Virginia, 5X. Fig. 2. Head and pronotum, dorsal. Fig. 3. Male sterna 7–9, ventral. Fig. 4. Male paraprocts, dorsal.

(n=5). Body length 9.6–10.0 mm (n=5). Head and pronotum pale yellow with contrasting pigmentation. General body color pale yellow with light brown to brown markings. (Fig. 1). Dorsal background of head pale yellow with medium brown bands connecting ocelli; bands widest anteriorly; bands truncate at anterior ocellus; triangular dusky spot anterior to anterior ocellus (Fig. 2). Antennal scape pale yellow with a diffuse diagonal brown stripe; flagellum segments 1–7 pale; remaining segments medium brown (Figs. 1–2). Pronotum with a wide medial pale stripe; rugosities irregular, raised, medium brown; anterolateral

margins medium brown; posterolateral margins pale (Fig. 2). Femora light brown dorsally, covered with uniformly spaced, short, brown setae; femora pale ventrally; tibia light brown, covered with uniformly spaced, short brown setae; tarsi medium brown (Fig. 1). Meso- and metanota light brown (Fig. 1). Wings hyaline, veins of forewing light basally, brown distally (Fig. 1). Terga light brown, posterior  $\frac{1}{3}$  medium brown; terga 1–8 with uniformly spaced brown setae. Sterna pale yellow to light brown; sternum 8 with a light brown recessed vesicle, 0.65X as wide as long; evenly rounded, extending to the posterior margin of sternum 8

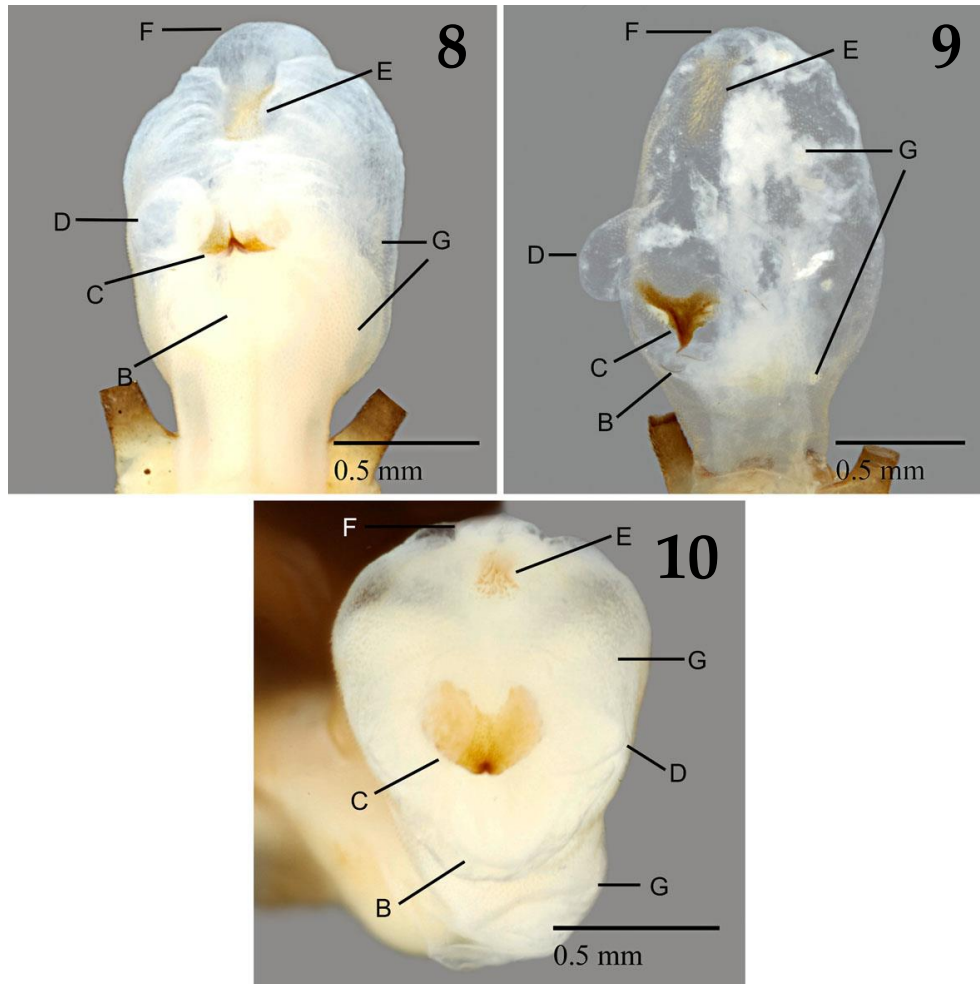




Figs. 5–7. Aedeagus of *Isoperla dewalti* sp. n., lateral, 5x. A. (vestigial) posteroventral membranous aedeagal tube, B. Posteroventral lobe, C. Posterior sclerotized arrowhead shaped aedeagal spine plate with upturned distal stout spine, D. Paired posterolateral lobes, E. Posteromedial rectangular patch of dense, stout, rust colored spines, F. Dorsal lobe, G. Dense short sharp spinulae, H. Anterior lobe. Fig. 5. Helton Creek, Grayson Co., Virginia. Fig. 6. Tributary to Le Conte Creek, Sevier Co., Tennessee. Fig. 7. Helton Creek, Grayson Co., Virginia.

(Fig. 3). Paraprocts short, triangular, slightly incurved, moderately sclerotized; trichoid sensilla scattered over dorsal surface, absent on apex; apex heavily sclerotized; tips acutely pointed (Fig. 4). Cerci brown, setose; cercal segments with a single, long, stout, ventral hair at posterior margin. Aedeagus with a long basal stalk, a highly reduced (vestigial) posteroventral membranous aedeagal tube, a posteroventral lobe, paired posterolateral

lobes, a dorsal lobe, and an anterior lobe (Figs. 5–10); posteroventral membranous aedeagal tube highly reduced (vestigial), appearing only occasionally as a small lobe at the base of the basal stalk (Fig. 5); aedeagus with a posteromedial rectangular patch of dense, stout, rust colored spines, and a single posterior sclerotized arrowhead shaped aedeagal spine plate with upturned distal stout spine (Figs. 8–10); apex of structure upturned



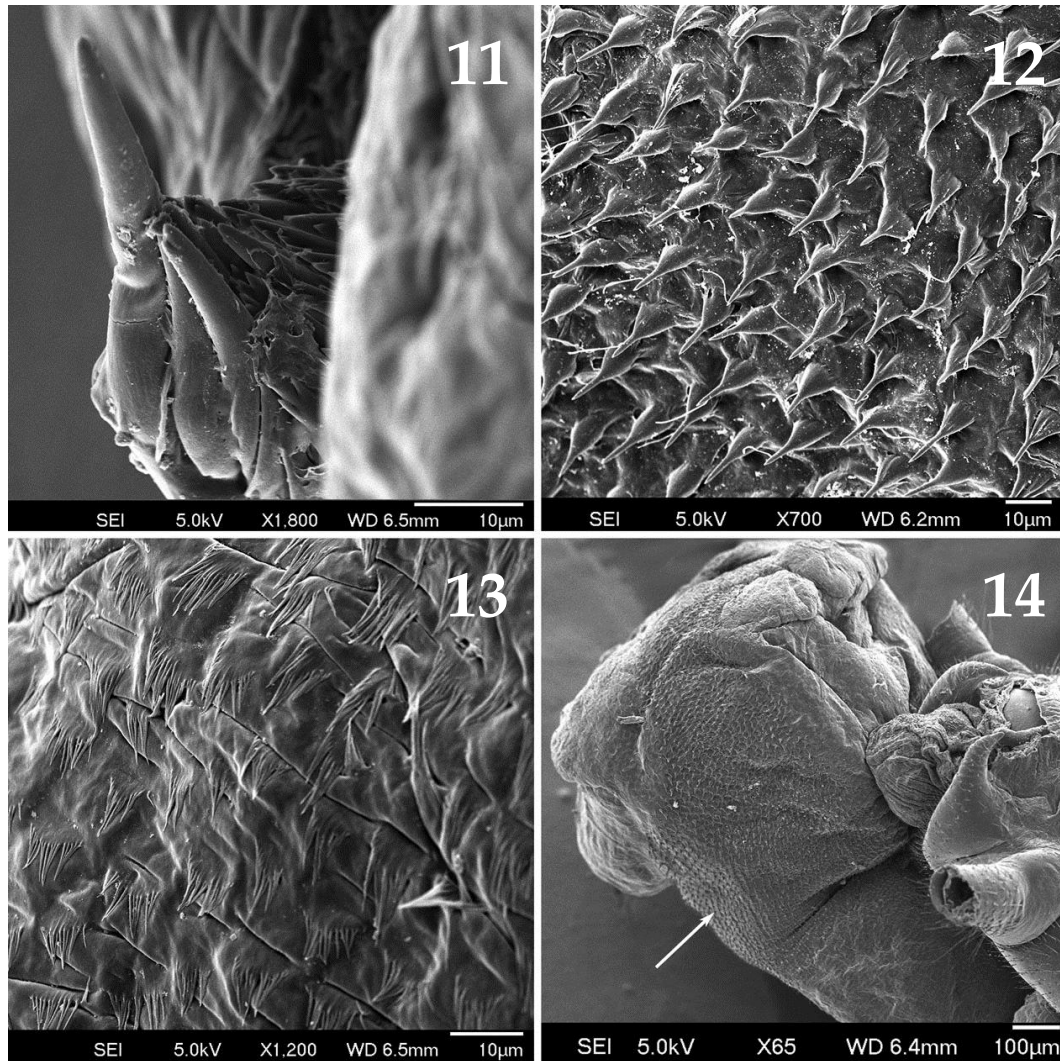
Figs. 8–10. Aedeagus of *Isoperla dewalti* sp. n., posterior, 5x. B. Posteroventral lobe, C. Posterior sclerotized arrowhead shaped aedeagal spine plate with upturned distal stout spine, D. Paired posterolateral lobes, E. Posteromedial rectangular patch of dense, stout, rust colored spines, F. Dorsal lobe, G. Dense short sharp spinulae, Fig. 8. Helton Creek, Grayson Co., Virginia. Fig. 9. Tributary to Le Conte Creek, Sevier Co., Tennessee. Fig. 10. Helton Creek, Grayson Co., Virginia.

with approximately 10 large stout rust colored spines (Fig. 11); base of plate with broad flat sclerotized plates with numerous small apical finger-like projections (Fig. 11); basal stalk with short sharp spinulae (Fig. 12); posterolateral lobes with sparse scale like plates medially; posteromedial area with shallow shelf-like scales with apical hair-like projections (Fig. 13); lateral area with dense short sharp spinulae (Fig. 14).

**Female.** Macropterous; forewing wing length 11.3–

12.6 mm (n=5). Body length 9.1–10.9 mm (n=5). Body coloration and morphology similar to male. Sternum 8 with variable subgenital plate extending approximately  $\frac{1}{3}$ – $\frac{1}{2}$  over sternum 9; subgenital plate broadly rounded (Fig. 15), or broadly triangular, truncate with shallow posteromedian emargination (Fig. 16), or broadly triangular (Fig. 17).

**Ovum.** Length 226.3–234.4  $\mu$ m (n=2); width 184.9–192.5  $\mu$ m (n=2). General shape oblong; cross section



Figs. 11–14. *Isoperla dewalti* sp. n., Helton Creek, Grayson Co., Virginia. Fig. 11. Posterior sclerotized arrowhead shaped aedeagal spine plate with upturned distal stout spine and broad flat sclerotized plates with numerous small apical finger-like projections, 1,800X. Fig. 12. Short sharp spinulae, 700X. Fig. 13. Shelf-like scales with apical hair-like projections, 1,200X. Fig. 14. Aedeagus, lateral, lateral area with dense short sharp spinulae (arrow) 65X.

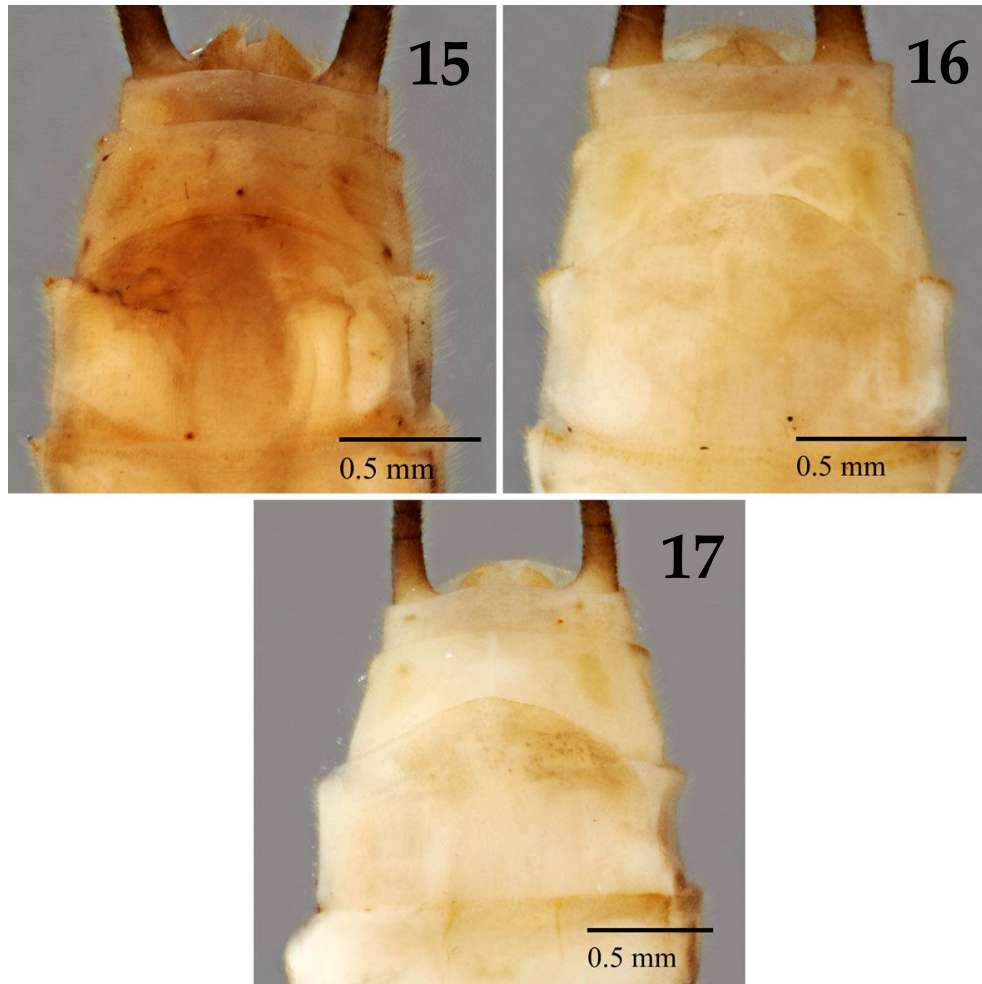
triangular, distinct cross shaped ridges dividing anterior and posterior poles; anterior end concave (Fig. 18). Color pale brown. Collar well-developed, flared apically with elevated longitudinal ridges; base not offset from egg body (Fig. 19). Hexagonal follicle cell impressions well-developed with thickened ridges and with numerous small mounds between reticulations (Figs. 18, 20). Eclosion line

absent, micropyles arranged singularly or in threes, positioned on top of cross ridges near anterior 1/3 of egg (Figs. 18, 20).

**Larva.** Unknown.

**Diagnosis.** *Isoperla dewalti* is placed in the *I. montana* Group based on the presence of a single posterior sclerotized arrowhead shaped aedeagal spine plate with an upturned distal stout spine, dark brown





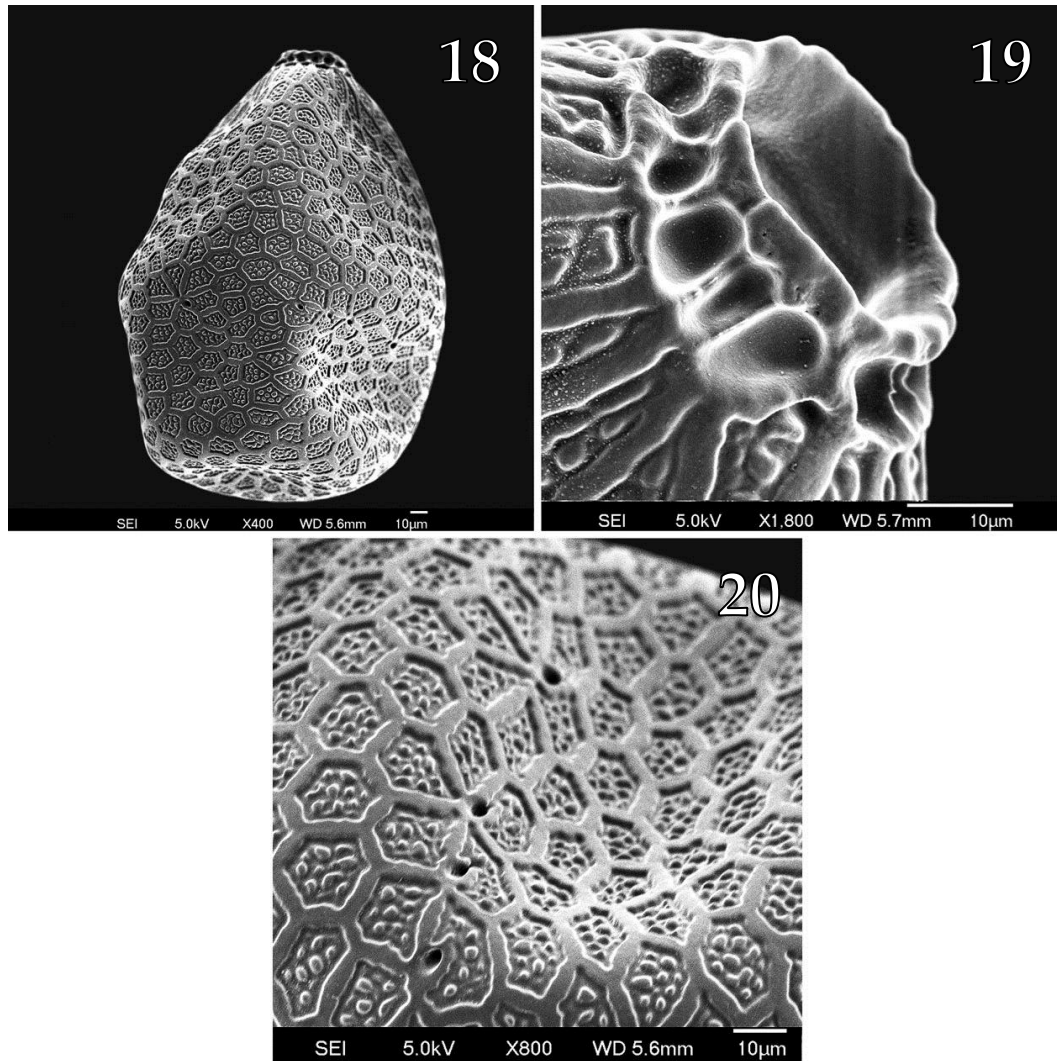
Figs. 15–17. Female subgenital plate of *Isoperla dewalti* sp. n., Helton Creek, Grayson Co., Virginia, 5X. Fig. 15. Broadly rounded Fig. 16. Broadly triangular, truncate with shallow posteromedian emargination. Fig. 17. Broadly triangular.

bars connecting the ocelli, and—albeit vestigial—a posteroventral membranous aedeagal tube. The new species is easily distinguished from *I. montana* and *I. nelsoni* by the lack of a well-developed posteroventral membranous aedeagal tube.

Several paratype specimens of *I. dewalti* that are deposited in the C. P. Gillette Museum of Arthropod Diversity had previously been identified as *I. montana*. The two species are superficially similar and it was likely thought that the posteroventral membranous aedeagal tube did not evert. However,

it is the experience of the authors that when a well-developed aedeagal tube is present it is the first structure to evert. Additionally, differences in the posteromedial patch of spines distinguish the two species. In *I. dewalti* the posteromedial patch of spines is broad, appearing as a uniform rectangular patch (Figs. 8–10). Whereas, in *I. montana* the posteromedial patch of spines is narrow, appearing as a thin ridge (figs. 33.15–33.16, Szczytko & Kondratieff 2015). Furthermore, *I. montana* possesses a dorsal rounded mound of dense, long,





Figs. 18–20. *Isoperla dewalti* sp. n., Tributary to Le Conte Creek, Sevier Co., Tennessee. Fig. 18. Ovum, 400X. Fig. 19. Ovum collar, 1,800X. Fig. 20. Ovum follicle cell impressions and micropyles, 800X.

stout, rust colored spines above the posteromedial patch of spines (figs. 33.15–33.16, Szczytko & Kondratieff 2015), which the new species lacks.

Females of the new species are most similar to *I. montana* and *I. kirchneri* Szczytko & Kondratieff, 2015. Females of the new species have a subgenital plate that extends approximately  $\frac{1}{3}$ – $\frac{1}{2}$  over sternum 9 and appears broadly rounded (Fig. 15), or broadly triangular, truncate with shallow posteromedian emargination (Fig. 16), or broadly triangular (Fig. 17), and have a dorsal head pattern with brown bands connecting the ocelli that are truncate at the

anterior ocellus with a triangular dusky spot anterior to the anterior ocellus (Fig. 2). Females of *I. montana* and *I. kirchneri* also have a subgenital plate that is variable, appearing broadly triangular usually with a shallow posteromedian emargination or pointed (figs. 25.5, 33.7, Szczytko & Kondratieff 2015) and have dorsal head patterns with brown bands connecting the ocelli. In *I. montana* the bands extend anteriorly to near the frons (occasionally pale brown or faint), and without a medium brown anterior patch near the frons (fig. 33.1, Szczytko & Kondratieff 2015). In *I.*



Fig. 21. Type locality. Helton Creek, Grayson Co., Virginia, USA.

*kirchneri* the bands sometimes extend anteriorly from the anterior ocellus, with a well-defined to diffuse spot on frons (fig. 25.1, Szczytko & Kondratieff 2015). The variation in subgenital plate morphology and dorsal head pattern exhibited by these species make the separation of isolated females very difficult.

Ova of the new species are similar to *I. kirchneri*, *I. montana*, *I. nelsoni*, and *I. signata* (Banks, 1902), all of which are oblong and triangular in cross section with distinct cross shaped ridges dividing anterior and posterior poles. Ova of the new species are distinguished from other similar ova by the inter-reticulate follicle cell impressions, which are developed as mounds in the new species (Figs. 18, 20) and punctations in the other similar species (figs. 25.19–25.22, 33.25–33.28, 36.9–36.11, 50.20–50.23, Szczytko & Kondratieff 2015).

**Biological notes.** There is no information about the biology or life cycle of *I. dewalti*. Based on the above records, the emergence period appears to be in early to mid-May, and as with most eastern *Isoperla*, a

univoltine life cycle is presumed. The type locality, Helton Creek, has a unique stonefly fauna, species with generally northern distributions (e.g. *Alloperla concolor* Ricker, 1936) and species with generally southern distributions (e.g. *Haploperla parkeri* Kirchner & Kondratieff, 2005) have been documented from this location. From the limited records available it is inferred that the new species also has a generally southern distribution, occurring in 1<sup>st</sup>–3<sup>rd</sup> order streams in the Piedmont Plateau and the southern Blue Ridge physiographic provinces (Fig. 22).

***Isoperla montana* (Banks, 1898)**

<http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:>

[TaxonName:468740](#)

Montane Stripetail

*Chloroperla montana* Banks, 1898:199. Holotype ♂ (MCZC), Mount Washington, Coos Co., New Hampshire.



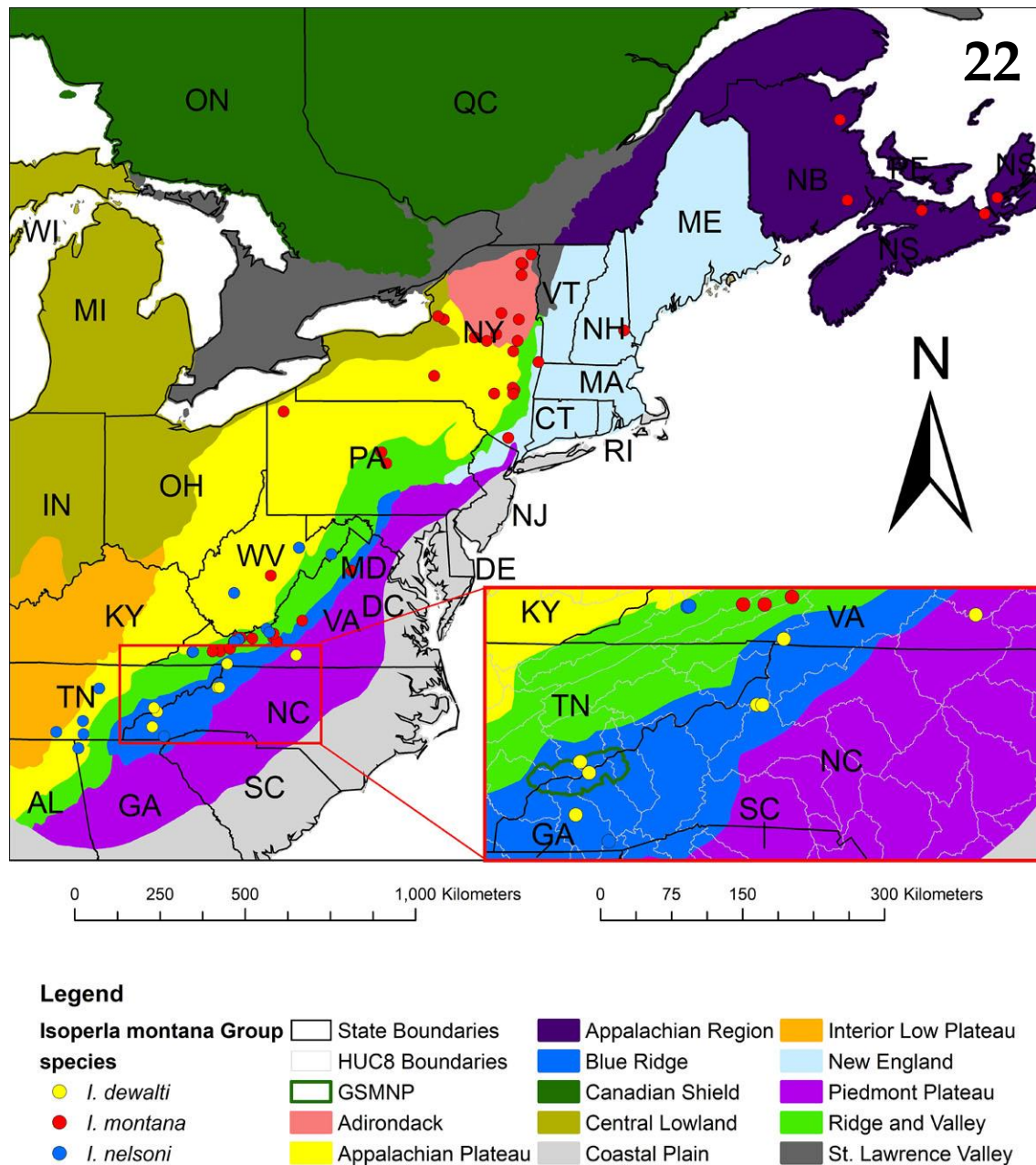


Fig. 22. Distribution map of examined material and physiographic provinces.

*Isoperla montana*: Banks 1906:175.

*Isoperla montana*: Illies 1966:410.

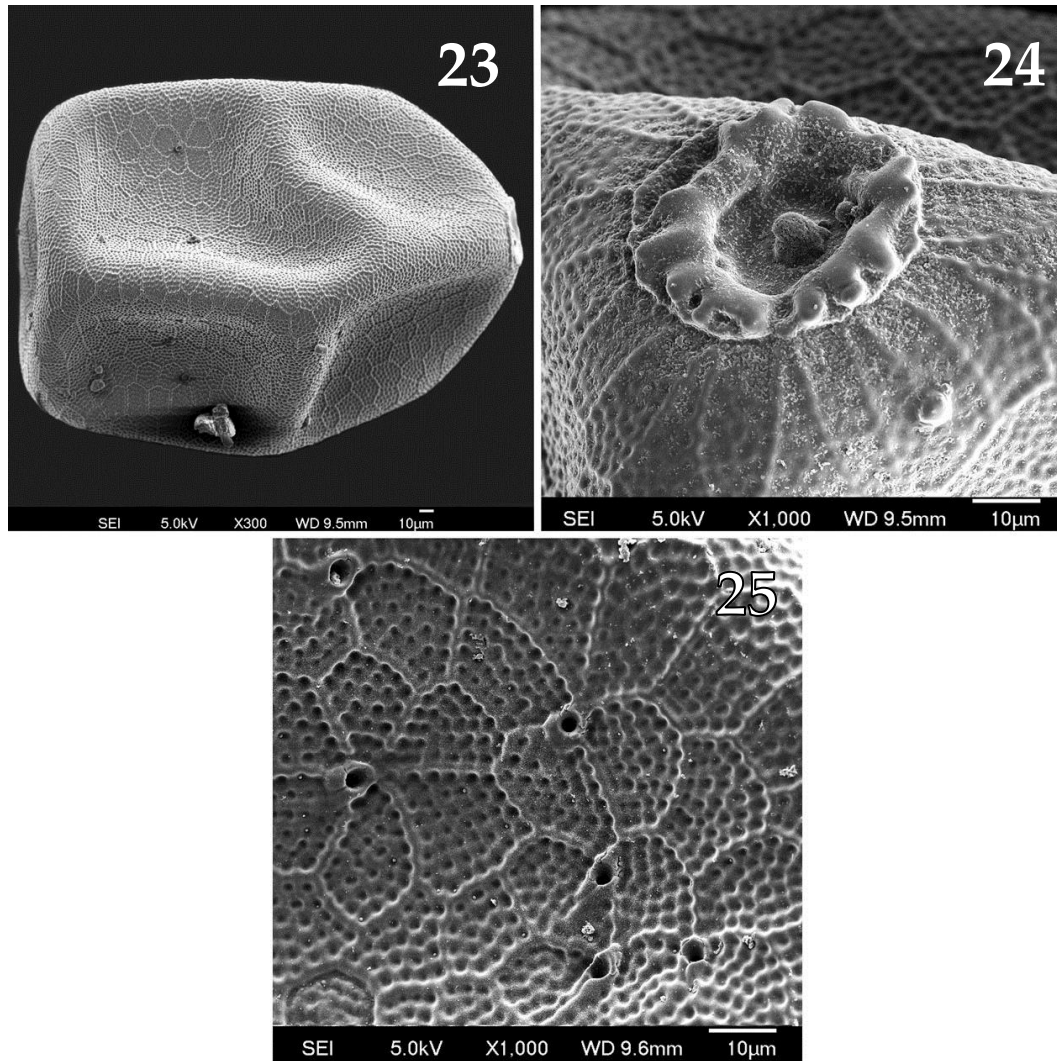
*Isoperla montana*: Zwick 1973:247, 293.

*Isoperla montana*: Szczytko & Kondratieff 2015:155.

**Material examined. U.S.A. – New York: Orange Co.**, spring trib. to Ramapo River, Rt. 17 N Arden Rd., N 41.27410, W 74.15320, 21 May 2008, L. Myers, B.C. Kondratieff, R.W. Baumann, 23♂, 3♀, 3N (CSUIC). **Oswego Co.**, Little Sandy Creek, Hwy 13, S Locana, [N 43.64889, W 76.006814], 17 June 1987, B.C. Kondratieff, R.W. Baumann, ♂, ♀ (CSUIC); Salmon River, CR 17, upstream of reservoir, N 43.58740, W 75.84780, 28 May 2008, L. Myers, B.C. Kondratieff, 2♂, 2♀ (CSUIC). **Rensselaer Co.**, Dill Brook, Dill Brook Rd., W Stillham, N 42.76300, W 73.35490, 19 April 2009 (emerged 28 April 2009), L. Myers, B.C. Kondratieff, 2♂, ♀ (CSUIC). **Saratoga Co.**, Alplaus Kill, Rt. 67, N 42.97420, W 74.01970, 26 May 2009, L. Myers, ♂ (CSUIC). **Ulster Co.**, Lucas Kill, Ralph Vedder Rd., [N 42.140024, W 74.022707], 21 May 2008, B.C. Kondratieff, 4♂, 3♀ (CSUIC). **Virginia: Montgomery Co.**, Mill Creek, Rt. 785, [N 37.26133, W 80.34061], 12 April 1979, R. Wallace, ♂ (CSUIC); North Fork Roanoke River, Rt. 603, [N 37.18667, W 80.352603], 18 May 1980, M. Spencer, ♂, 2♀ (CSUIC). **Russell Co.**, Loop Creek, Rte 619, [N 36.890811, W 81.953779], 12 April 1980, B.C. Kondratieff, ♂ (CSUIC). **Smyth Co.**, North Fork Holston River, Rt. 42, Saltville, [N 36.89084, W 81.749537], 20 May 1993, B.C. Kondratieff, R.F. Kirchner, ♀ (CSUIC). **Tazewell Co.**, headwaters Station Spring, MBC Ranch off Rt. 666, Burkes Garden, [N 37.094939, W 81.38785], 8 May 1982, B.C. Kondratieff, 4♂, 3♀ (CSUIC); Station Spring Creek, MBC Ranch, [N 37.100225, W 81.370093], 28 May 1992, B.C. Kondratieff, 5♂, 8♀ (CSUIC); Same location, 17 May 1994, B.C. Kondratieff, R.F. Kirchner, 8♂, 7♀ (CSUIC); Station Spring Creek, Rt. 623, Moore Bros. Farm, [N 37.104181, W 81.346915], 18 May 1979, B.C. Kondratieff, 10♂, 18♀ (CSUIC). **West Virginia: Webster Co.**, Back Fork Elk River, Webster Spring, [N 38.480435, W 80.413394], 9 May 1980, J. Hill, ♂, ♀ (CSUIC). **New Records. Canada – New Brunswick: Albert Co.**, Kennebecasis River, Rte 895, [N 45.826391, W

65.205347], 3 June 1998, B.C. Kondratieff, R.W. Baumann, 3♀ (CSUIC). **Northumberland Co.**, Big Eskedellocc River, Hwy 8, [N 47.28819, W 65.405769], 17 June 1999, B.C. Kondratieff, R.W. Baumann, ♂ (CSUIC). **Nova Scotia: Antigonish Co.**, Tracadie River, Hwy 16, S Monastery, [N 45.576041, W 61.586629], 22 June 1993, B.C. Kondratieff, R.W. Baumann, ♂ (CSUIC). **Colchester Co.**, Baileys Brook, Rt. 256, W The Falls, [N 45.640165, W 63.250553], 3 June 1998, B.C. Kondratieff, R.W. Baumann, ♂, 6♀ (CSUIC). **Iverson Co.**, N of Melford, Glenn Brook, Hwy 105, [N 45.877216, W 61.25576], 11 June 1998, B.C. Kondratieff, R.W. Baumann, ♂ (CSUIC). **U.S.A. – New Hampshire: Strafford Co.**, Mad River, 2 km SW Farmington, [N 43.37762, W 71.093572], 5 June 2011, D.S. Chandler, ♂, ♀ (CSUIC). **New York: Clinton Co.**, spring, Recore Rd., N 44.81860, W 73.54110, 16 March 2009 (emerged 15 April 2009), L. Myers, ♂, ♀ (CSUIC); trib. to True Brook, Clark Hill Rd., N 44.65880, W 73.80220, 1 June 2007, J. Mihuc, ♂ (CSUIC); Same location, 13–18 June 2008, J. Mihuc, ♀ (CSUIC); Same location, 30 May–8 June 2008, J. Mihuc, ♂, ♀ (CSUIC); True Brook, True Brook Rd., [N 44.63703, W 73.769705], 16 May 2006, L. Myers, ♀ (CSUIC). **Cortland Co.**, Gridley Creek, Rt. 392 nr. East Virgil, N 42.4944, W 76.1062, 19 February 2008, L. Myers, ♂, 2♀ (CSUIC). **Delaware Co.**, spring-fed trib. to Emory Brook, Rt. 28 Fleischmanns, N 42.15110, W 74.52240, 27 May 2009, L. Myers, B.C. Kondratieff, ♀ (CSUIC). **Essex Co.**, Pettigrew Brook, Bonnieview Rd. nr. Willington, [N 44.43083, W 73.800231], 26 June 2007, L. Myers, B.C. Kondratieff, ♂, 2♀ (CSUIC). **Greene Co.**, [Shingle Kill], Maple Lawn Rd., [N 42.265848, W 74.032405], 24 June 2007, L. Myers, B.C. Kondratieff, ♂, ♀ (CSUIC); Kiskatom Brook, Rt. 32 nr. Kiskatom, N 42.22170, W 73.98720, 26 May 2009, L. Myers, B.C. Kondratieff, ♀ (CSUIC). **Saratoga Co.**, small stream, Alpine Meadows Rd., N 43.17860, W 73.90450, 12 February 2009 (emerged 20 April 2009), L. Myers, ♂ (CSUIC). **Warren Co.**, Glen Creek, Glen Creek Rd., N 43.58510, W 73.87130, 15 May 2008, L. Myers, E. White, ♀ (CSUIC). **Pennsylvania: Clinton Co.**, Fishing Creek, Sieg Conference Center, Marrow Rd., [N 40.988602,





Figs. 23–25. *Isoperla smithi*, paratype, 3 mi. NE Mathias, 38°55'N, 78°49'W, Hardy Co., West Virginia. 23. Ovum, 300X. 24. Ovum collar, 1,000X. 25. Ovum follicle cell impressions and micropyles, 1,000X.

W 77.497564], 8 June 2013, B.C. Kondratieff, J. Sandberg, 27♀ (CSUIC); Fishing Creek, Sieg Conference Center, Marrow Rd., [N 40.988602, W 77.497564], 9 June 2013, B.C. Kondratieff, J. Sandberg, 3♂ (CSUIC). **Crawford Co.**, trib. to French Creek, Hwy 19, 1 mi. S Cambridge Springs, [N 41.796281, W 80.077902], 17 April 1979, S.W. Szczytko, K.W. Stewart, ♂, 2♀ (CSUIC). **Mifflin Co.**, Honey Creek, Reeds Gap State Park, Lancaster Valley Rd., [N 40.766351, W 77.370555], 7 June 2013, B.C. Kondratieff, J. Sandberg, 2♀ (CSUIC). **Virginia: Bland Co.**, Kimberling Creek, Hwy 42, Rte 606, N

37.16646, W 80.90034, 7 May 2017, C. Verdone, B.C. Kondratieff, S. Roble, ♂ (CSUIC); Wolf Creek, Grapefield Rd., Stephen Levitt Property, N 37.14702, W 81.26314, 4 June 2016, C. Verdone, B.C. Kondratieff, 3♂, 4♀ (CSUIC); Same location, 6 June 2016, C. Verdone, B.C. Kondratieff, 5♂, 6♀ (CSUIC); Same location, 8 June 2016, C. Verdone, B.C. Kondratieff, 3♀ (CSUIC); Same location, 23 May 2017, C. Verdone, ♂, 2♀ (CSUIC); Same location, 25 May 2017, C. Verdone, D. Fuller, ♂, 2♀ (CSUIC); Same location, 27 May 2017, C. Verdone, D. Fuller, ♂ (CSUIC); Wolf Creek, Rte 614, Grapefield Rd.,

Wolf Creek Picnic Area, N 37.18026, W 81.19496, 7 May 2017, C. Verdone, B.C. Kondratieff, S. Roble, 2♀ (CSUIC). **Botetourt Co.**, North Creek, Jefferson National Forest, [N 37.541442, W 79.586821], 27 April 1981, J. Widlack, ♀ (CSUIC). **Montgomery Co.**, Mill Creek, Rt. 785, [N 37.26133, W 80.34061], 4 April 1978 (emerged 15 April 1978), B.C. Kondratieff, 2♂, 3♀ (CSUIC); Same location, 15 April 1980, B.C. Kondratieff, ♀ (CSUIC); Same location, 26 April 1980, B.C. Kondratieff, 2♂, 7♀ (CSUIC); North Fork Roanoke River, Rt. 603, [N 37.18667, W 80.352603], 30 April 1978, B. C. Kondratieff, ♀ (CSUIC); small trib. to South Fork Roanoke River, Rte 637, [No GPS], 5 June 1981, A. Beck, 25♀ (CSUIC). **Rappahannock Co.**, Rocky Run, Rt. 707, [N 38.577655, W 78.28196], 7 July 1979, Firth, ♀ (CSUIC). **Smyth Co.**, North Fork Holston River, Co. Rd. 620, jct. Co. Rd. 716, [N 36.94807, W 81.49062], 27 May 1992, B.C. Kondratieff, ♂, 2♀ (CSUIC); Same location, 20 May 1993, B.C. Kondratieff, ♂, 5♀ (CSUIC); Same location, 16 May 2004, B.C. Kondratieff, R.F. Kirchner, R.E. Zuellig, ♂, ♀ (CSUIC). **Tazewell Co.**, Cove Creek, Rte 662, off Hwy 61, 37.17837, W 81.29900, 6 June 2016, C. Verdone, B.C. Kondratieff, ♂ (CSUIC); Same location, 7 May 2017, C. Verdone, B. Kondratieff, S. Roble, 4♂, 10♀ (CSUIC); spring nr. spring house, Cove Creek, Rte, N 37.17989, W 81.30067, 7 May 2017, C. Verdone, B. Kondratieff, S. Roble, 2♀ (CSUIC).

**Distribution.** Canada – NS, ON, PQ. U.S.A. – CT, DE, IN, KY, ME, MN, NC, NH, NJ, NY, OH, PA, VA, WV (Szczytko & Kondratieff 2015).

**Biological notes.** *Isoperla montana* is the most widespread species of the *I. montana* Group occurring from New Brunswick, Canada to North Carolina and west to Indiana (Szczytko & Kondratieff 2015). The majority of Virginia records are from the Ridge and Valley Physiographic Province with few records east of this province, particularly in the southern part of the state. Szczytko and Kondratieff (2015) reported *I. montana* from North Carolina based on two females collected by the authors (CJV, BCK). As noted above, species determination of isolated females is difficult, especially in the southern Appalachians where the ranges of *I. montana* and *I. kirchneri* overlap, as such

this record should be regarded as tentative. In addition to the material listed above, we also examined specimens of *I. montana* reported by Grubbs (2016) from Alabama. These specimens appear to represent an undescribed taxon based on details of the aedeagus and ovum, but more specimens with the aedeagus everted and mature ova are needed for confirmation.

#### *Isoperla nelsoni* Szczytko & Kondratieff, 2015

<http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:>

[TaxonName:468741](#)

Nelson Stripetail

*Isoperla nelsoni* Szczytko & Kondratieff, 2015:174. Holotype ♂ (USNM), Clear Creek, Morgan Co., Tennessee.

*Isoperla smithi* Szczytko & Kondratieff, 2015:250. Holotype ♂ (USNM), 3 mi. NE Mathias, 38°55'N, 78°49'W, Hardy Co., West Virginia. **Syn. n.**

**Material examined. U.S.A. – Georgia: Dade Co.**, [Daniel Creek], Rt. 136, 9.1 mi. W jct. 176/193, just past entrance Cloudland State Park, [N 34.816158, W 85.491197], 8 May 1980, C.H. Nelson, 3♂, ♀ (CSUIC, paratypes). **North Carolina: Macon Co.**, Bridal Veil Falls, nr. Highlands, [N 35.071766, W 83.229483], 8 May 2000, B. Stark, B. Bryan, ♂ (CSUIC, paratype). **Tennessee: Hamilton Co.**, Shoal Creek, Shoal Creek Rd. off Rt. 127, Signal Mountain, [N 35.117311, W 85.353965], 29 May 1981, C.H. Nelson, 3♂, 3♀ (CSUIC, paratypes). **Sequatchie Co.**, Brush Creek, N Dunlap, [N 35.404253, W 85.369517], 12 May 2000, B.C. Kondratieff, R.F. Kirchner, ♂ (CSUIC, paratype). **Virginia: Montgomery Co.**, small trib. Craig Creek, 0.6 mi. E Hwy 460, [N 37.29070, W 80.45221, 1 May 1992, B.C. Kondratieff, 4♂, 7♀ (CSUIC, paratypes, *I. smithi*); spring-fed stream, Rte 621, ~0.5 off State Rte 460, [N 37.29070, W 80.45221], 29 May 1979, B.C. Kondratieff, 5♂ (CSUIC). **Tazewell Co.**, springs into Station Spring Creek, Burkes Garden, [N 37.097691, W 81.373222], 18 May 1979, B.C. Kondratieff, 2♂ (CSUIC, paratypes); **West Virginia: Hardy Co.**, Lower Cove Run, 3 mi. NE of Mathias, N 38.916667, W 78.816667, 16–31 May 2000, D. Smith, 4♂, 7♀ (CSUIC, paratypes, *I. smithi*). **Kanawha Co.**, Buzzard Branch, I-64/77, [N 38.11827, W 81.38546], 16 May 1994, B.C.

Kondratieff, R.F. Kirchner, 2♀ (CSUIC, paratypes, *I. smithi*).

**New Records. Kentucky: Rowan Co.,** Triplett Creek, 0.4 mi. N of I-64, Conley Rd., (No GPS), 25 May 1994, B.C. Kondratieff, R.F. Kirchner, 1♂ (CSUIC). **Tennessee: Cumberland Co.,** 3 mi. N of Adams Bridge Obed River, N 36.10321, W 84.94164, 22 May 1994, B. C. Kondratieff, R.F. Kirchner, 2♂ (CSUIC, paratypes). **Franklin Co.,** seeps on Hwy 64, 4 mi. E Cowan, [N 35.167581, W 86.074139], 28 May 1994, B. Stark, ♂, ♀ (CSUIC). **Virginia: Bland Co.,** Wolf Creek, Rte 614, Stephen Levitt Property, N 37.14702, W 81.26314, 25 May 2017, C. Verdone, D. Fuller, ♂, ♀ (CSUIC). **Giles Co.,** spring, Rte 613 NE side of Mountain Lake, N 37.37012, W 80.52918, 28 May 2016, C. Verdone, B.C. Kondratieff, ♂, ♀ (CSUIC). **Scott Co.,** Laurel Branch, FR 700, > 1km from FR 701, N 36.87556, W 82.48100, 6 May 2017, C. Verdone, B.C. Kondratieff, S. Roble, ♂ (CSUIC); Laurel Branch and trib., FR 700, N 36.87844, W 82.46630, 6 May 2017, C. Verdone, B.C. Kondratieff, S. Roble, 4♂ (CSUIC). **Tazewell Co.,** Station Spring Creek, MBC Ranch Rd., Burkes Garden, [N 37.100362, W 81.370206], 17 May 1994, B.C. Kondratieff, R.F. Kirchner, 2♂ (CSUIC). **West Virginia: Tucker Co.,** Fernow Experimental Forest, [N 39.054807, W 79.669890], 20 June 1990, emergence trap, DPE, 4♂, 4♀ (CSUIC); Same location, 16–23 May 1990, emergence trap DPE, 2♀ (CSUIC); Same location, 20–26 July 1990, emergence trap, DPE, 1♂ 2♀ (CSUIC); Same location, 21 May–5 June 1990, emergence trap, DPE, 1♂ (CSUIC).

**Distribution.** U.S.A. – AL, GA, KY, NC, TN, VA, WV (Szczytko & Kondratieff 2015).

**Biological notes.** The paratype series of *I. smithi* from the type locality included a male with the aedeagus everted and females with mature ova, which was previously unknown. These specimens were examined and were determined to be conspecific with *I. nelsoni* based on details of the aedeagus, female subgenital plate and ova, which were examined using SEM (Figs. 23–25). With the synonymy of these species the range of *I. nelsoni* is extended northward to include locations in Hardy, Kanawha, and Tucker counties, West Virginia. *Isoperla nelsoni* occurs in pristine springs to medium–large streams from the Interior Low

Plateau of south central Tennessee, east to the Blue Ridge in North Carolina and north to the Appalachian Plateau of West Virginia. Although uncommonly collected, *I. nelsoni* may be more common than records indicate given the wide range of habitats this species can occur.

## DISCUSSION

In the *I. montana* Group some aedeagal characters can be observed through the cuticle such as the posteroproximal sclerotized arrowhead-shaped spine plate. However, as noted by Szczytko and Kondratieff (2015), the aedeagus can be twisted in situ, obscuring the actual shape of the spine plate. Several specimens previously identified as *I. montana* were cleared in a 10% KOH solution and the aedeagus everted using a pipette. What had initially appeared as a single spine were actually two, revealing the true identity of the specimens as *I. kirchneri*. Given this potential for misidentification, species determinations should only be made with specimens with the aedeagus everted.

Eversion of un-everted, preserved specimens is possible with varying degrees of success by clipping the abdomen, soaking it in 10% KOH and heating the solution to a near boil. Once the tissue is sufficiently softened it can be removed using a modified, size 0 insect pin with the tip recurved. Then, the aedeagus can be everted by lifting the 10<sup>th</sup> abdominal segment dorsad and rolling a fingertip from the cut end towards the abdomen apex inflating the aedeagus like a balloon. Or a fine tipped, disposable pipette can be placed on the membrane between the 9<sup>th</sup> and 10<sup>th</sup> tergum and the aedeagus can be suctioned out. A note of caution when using a pipette, tissue adhered to the aedeagal structure can rupture the fragile aedeagal membrane if not sufficiently dissolved.

Success of these methods are dependent on the age and size of the specimen. Fresh specimens > 8 mm produce the best, most consistent results, but eversion is possible with older specimens. Extra careful handling of older specimens is generally required to avoid cracking the abdominal cuticle. Additionally, older specimens may require iterative



baths in KOH to soften remnant tissue, which can become quite hard over time.

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#### REFERENCES

- Beaty, S.R., V.B. Holland, & D.R. Lenat. in press. *Isoperla arcana* and *Isoperla borisi* (Plecoptera: Perlodidae), two new stonefly species from North Carolina, U.S.A. with notes on the distribution of *Isoperla powhatan*. submitted to *Illiesia*.
- DeWalt, R.E., M.D. Maehr, U. Neu-Becker & G. Stueber. 2017. Plecoptera Species File Online. Version 5.0/5.0. Accessed 29 September 2017. <http://Plecoptera.SpeciesFile.org/>
- Grubbs, S.A. 2016. Taxonomic notes on the eggs of eastern Nearctic *Isoperla* (Plecoptera: Perlodidae: Isoperlinae). *Illiesia*, 12(08):35–41. <http://illiesia.speciesfile.org/papers/Illiesia12-08.pdf>
- Szczytko, S.W. & B.C. Kondratieff. 2015. A review of the eastern Nearctic Isoperlinae (Plecoptera: Perlodidae) with the description of twenty-two new species. Monographs of *Illiesia*, Number 1:1–289. <http://illiesia.speciesfile.org/papers/Monographiae-of-Illiesia.pdf>
- Verdone, C.J. & B. Kondratieff. 2016. A new species *Isoperla* Banks (Plecoptera: Perlodidae) from the Appalachian Mountains, Virginia & West

Virginia, U.S.A. *Illiesia*, 12(13):74–85. <http://illiesia.speciesfile.org/papers/Illiesia12-13.pdf>

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